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New Drugs

Obinutuzumab in hematologic malignancies: Lessons learned to date

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ABSTRACT

The routine use of anti-CD20 monoclonal antibodies (mAbs) has improved patient outcomes in CD20-positive non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL). Despite the clinical success achieved with rituximab, relapses are still common with further improvements in anti-CD20 mAb efficacy required. Many novel anti-CD20 antibodies are in development, but obinutuzumab is currently the only type II glycoengineered anti-CD20 mAb in clinical testing.

Obinutuzumab has increased antibody-dependent cell-mediated cytotoxicity, reduced complement-dependent cytotoxicity and enhanced direct non-apoptotic cell death. In preclinical models, obinutuzumab induced superior tumor remission compared with rituximab at the equivalent dose levels, and was active in rituximab-refractory tumors. Obinutuzumab exhibits encouraging efficacy as monotherapy in NHL, and combined with chemotherapy in relapsed/refractory NHL and treatment-naïve symptomatic CLL. In a recent randomized, phase III trial in patients with untreated comorbid CLL, overall response rate was significantly greater (78% *vs.* 65%, $P < 0.0001$) and median progression-free survival was significantly prolonged (26.7 *vs.* 15.2 months, $P < 0.0001$) for obinutuzumab plus chlorambucil *vs.* rituximab plus chlorambucil.

Obinutuzumab is a type II anti-CD20 antibody that utilizes distinct mechanisms of action relative to type I antibodies like rituximab and has led to significant clinical improvement over rituximab in a phase III trial in CLL. Further trials are ongoing to determine whether such improvements in outcome will be seen in CD20-positive B-cell malignancies.

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Introduction

The anti-CD20 monoclonal antibody (mAb) rituximab (Rituxan®, MabThera®) has improved clinical outcomes for patients with a broad range of B-cell malignancies. Phase II trials demonstrated single-agent activity and durable clinical responses with rituximab, leading to health authority approval in the US in 1997, and in the EU in 1998. However, it was not until randomized phase III trials of combined rituximab with chemotherapy showed an improvement in overall survival (OS), that this became the standard of care in B-cell non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) [1–7]. However, with up to 40% of diffuse large B-cell lymphoma (DLBCL) patients still dying of lymphoma, and most patients with follicular lymphoma (FL) or CLL relapsing and eventually developing chemotherapy- and rituximab-refractory disease, there remains room for improvement [8,9]. Given rituximab's success, the development of anti-CD20 mAbs with enhanced or novel effector mechanisms may yield improved efficacy and/or show activity in rituximab-refractory patients. This article discusses the preclinical and emerging clinical trial data using obinutuzumab (GA101; GAZYVA®, GAZYVARO®).

How do structural components define type I and type II anti-CD20 mAb activity?

Anti-CD20 mAbs are classified by their CD20-binding characteristics, ability to induce complement-dependent cytotoxicity (CDC),

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Table 1
Summary of functional differences between type I and type II mAbs.

Type I mAbs (rituximab, ofatumumab, veltuzumab, ublituximab)	Type II mAbs (obinutuzumab, tositumomab)
Localization of CD20 into lipid rafts, increasing CDC	No localization of CD20 into lipid rafts, which leads to reduced CDC
No homotypic adhesion, low cell death/apoptosis	Homotypic adhesion, resulting in noncaspase-dependent direct cell death
Full CD20 binding capacity at saturating conditions	Half-maximal CD20 binding at saturating conditions, stimulating greater levels of apoptotic induction than type I mAbs
CD20 modulation	Less or no CD20 modulation
Induce ADCC	
Induce ADCP	

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; CDC, complement-dependent cytotoxicity; mAbs, monoclonal antibody.

Table 2
Functional comparison of Fc- or glycoengineered anti-CD20 mAbs to rituximab.

Name (INN)	Company	ADCC	CDC	Cell death
Rituximab	Roche	+	+	+
Obinutuzumab [23]	Roche	+++	+/−	+++
Ocaratuzumab [15]	Lilly	+++	+	+
PRO131921 [111]	Genentech	+++	+	+
Ublituximab [17,43]	LFB/TG Therapeutics	+++	+	+
KM3065 [112,113]	Kyowa Hakko Kirin	+++	+	+

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; mAbs, monoclonal antibodies; +, activity induced by rituximab; ++ and +++, increased activity compared with rituximab; −, reduced activity compared with rituximab.

and immune effector cell effects (Table 1) [10–14]. Most anti-CD20 mAbs investigated are of type I (rituximab, ofatumumab, ublituximab, veltuzumab, ocaratuzumab; Table 2) [15–19], and binding to CD20 on lymphoma cells induces rapid translocation of anti-CD20 mAb–CD20 antigen complexes into lipid rafts (Fig. 1A) [13]. This complex formation leads to strong CDC, but only weak direct apoptosis (cell death) [10–12,20,21]. Type I and type II mAbs both induce antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) through immune effector cell interactions [22]. Conversely, type II mAbs (tositumomab, obinutuzumab) potently induce direct cell death but do not localize mAb–CD20 antigen complexes into lipid rafts (Fig. 1B), resulting in low levels of CDC [12,13,23]. CDC induction by obinutuzumab is >10- to 100-fold less than with the type I mAbs rituximab and ofatumumab [24], resulting in a further-increased capacity to bind and activate natural killer (NK) cells in the presence of complement [25]. FcγRIIb-mediated CD20 internalization has been implicated in reduced rituximab efficacy. Conversely, type II CD20 antibodies result in reduced FcγRIIb-induced CD20 internalization, which may further enhance immune effector function [26].

Antibody activity may be manipulated firstly by changing the target CD20 epitope bound by the mAb or secondly by altering the Fc region to enhance immune effector cell activity (ADCC, ADCP). For example, compared with rituximab, ofatumumab exhibits increased CDC by binding to a different CD20 epitope [24]. Fc alterations are illustrated by ocaratuzumab (AME-133v), which exhibits increased ADCC via an amino acid substitution in the Fc domain that alters Fcγ receptor (FcγR) interaction [27].

Enhancing the direct effects mediated by type II CD20 antibodies

Compared with rituximab, obinutuzumab firstly targets a different, but overlapping, epitope of the CD20 extracellular domain [14]. The selection of a particular valine-for-leucine substitution in the elbow hinge region of obinutuzumab appears to have considerable impact on its in vitro activity (Fig. 2). These two modifications result in increased in vitro direct cell death induction, demonstrated in various tumor cell lines (FL, mantle cell lymphoma [MCL], DLBCL, CLL) [23,28,29], most likely by affecting the flexibility and angle of antibody binding to CD20.

Obinutuzumab appears to cause cell death via homotypic aggregation (the clustering of lymphoma cells by bound antibody), which proceeds through a caspase-independent, nonapoptotic, lysosome-mediated mechanism involving reactive oxygen species [23,28–33]. This mechanism is independent of effector cell engagement and has also been reported for other antigens such as e.g. HLA-DR [34] or CD37 [35], which may be relevant in patients with impaired immunity.

Enhancing Fc effector function

The oligosaccharide composition of the antibody Fc portion affects its affinity for FcγRIII on the immune effector cell surface (NK cells, neutrophils, macrophages/monocytes) [36]. Glycoengineering the carbohydrate moiety of obinutuzumab

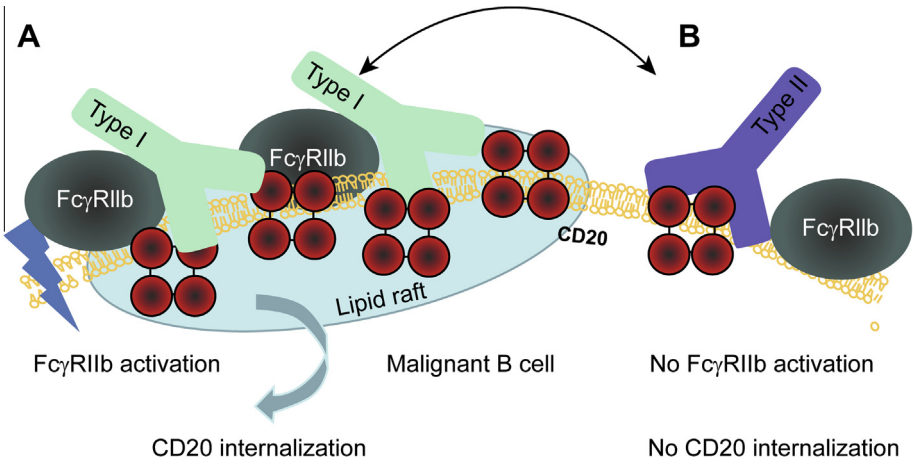

Fig. 1. The effects of type I and type II anti-CD20 mAb binding to CD20 on FcγRIIb internalization. (A) There is potential for type I mAbs, like rituximab, to bind not only to CD20 but also to FcγRIIb. Simultaneous engagement of FcγRIIb could trigger internalization of antibody via lipid rafts. (B) In contrast, type II antibodies, such as obinutuzumab, do not generally engage CD20 in a manner that would permit simultaneous FcγRIIb binding. Consequently, internalization of type II mAbs via lipid rafts is comparatively reduced. Adapted from MAbs 5:22-33, 2013; ©2013 Landes Bioscience [13]. mAb, monoclonal antibody.

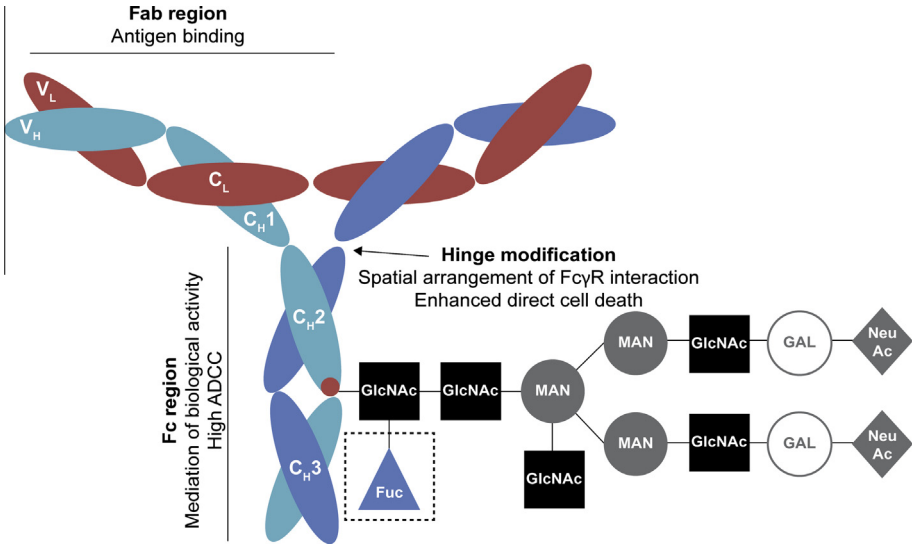

Fig. 2. Structure of obinutuzumab and the Fc-attached glycan tree modified by glycoengineering. The illustration shows the main peptide chains of obinutuzumab that compose the Fab antigen-binding domain (V_H, V_L, C_H1, and C_H) and the Fc domain (C_H2 and C_H3) which interacts with FcγRs. The glycan tree is attached to asparagine 297. The core fucose that is not added to the glycan tree is highlighted with a dashed box. ADCC, antibody-dependent cell-mediated cytotoxicity, C_H, constant heavy chain; C_L, constant light chain; Fuc, fucose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; Man, mannose; NeuAc, *N*-acetylneuraminic acid; V_H, variable heavy chain; V_L, variable light chain.

resulted in a lower fucose content than conventional IgG antibodies like rituximab (Fig. 2). Defucosylated moieties have increased affinity for FcγRIIIa and FcγRIIIb, and interact more effectively with FcγRIII-expressing effector cells [37–40], increasing recruitment capacity and activation to ultimately improve in vitro ADCC and ADCP compared with fully fucosylated antibodies [23,41,42]. Consequently, antibodies like obinutuzumab or ublituximab induce NK cell-mediated ADCC to a greater extent than rituximab or ofatumumab, with similar levels of ADCP [17,24,43]. However, in the presence of physiologic levels of immunoglobulins, monocyte/macrophage-mediated phagocytosis (ADCP) and ADCC are enhanced [42]. Furthermore, obinutuzumab activates neutrophils and mediates phagocytosis through FcγRIIIb more efficiently than rituximab [41]. Glycoengineering also appears to overwrite inhibition by inhibitory KIR receptors [44].

Preclinical observations with obinutuzumab

In normal and malignant B-cell lines and xenograft models, obinutuzumab induced superior activity to rituximab, even under rituximab-saturating dose/equivalent exposure conditions [23]. In a DLBCL xenograft model progressing under rituximab treatment, tumors did not respond to further rituximab, but progression was delayed and tumor volume reduced with obinutuzumab. Obinutuzumab-chemotherapy combinations also prolonged survival to a greater extent than rituximab combinations in a mouse MCL model [23,45].

In a cynomolgus monkey model, rituximab and obinutuzumab both induced complete peripheral blood B-cell depletion, whereas obinutuzumab induced significantly greater lymphoid and splenic B-cell depletion [23]. Similarly, obinutuzumab induced greater levels of B-cell depletion than rituximab in whole blood from healthy volunteers [23,24], and blood from patients with CLL [46,47].

What are the potential clinical implications of the different mechanisms of action of obinutuzumab?

Resistance mechanisms to type I anti-CD20 mAbs are incompletely understood. Contributing factors include intrinsic tumor cell alterations (e.g., loss of CD20 from the lymphoma cell surface, as observed in rare rituximab-refractory patients) [29], and host immunologic environment [48]. Tumor cell resistance to apoptosis may predict impaired response/resistance to chemotherapy and immunochemotherapy. Fc–FcγR interaction between immune effector cells and CD20-bound antibody is essential to induce antibody-dependent cell killing mechanisms including ADCC and ADCP [49]. Both activating and inhibitory FcγRs modulate the cytotoxicity of rituximab against tumors in mice [50]. In normal mouse B cells [51,52], and adoptively transferred primary murine lymphoma [53] from syngeneic mouse models, FcγRs are required for anti-CD20-mediated B-cell depletion. FcγR polymorphisms also appear to be clinically important; a polymorphism at residue 158 that substitutes valine for phenylalanine increases affinity for mAbs, and is associated with higher response rates in FL patients receiving rituximab monotherapy [54]. The significance is less clear for immunochemotherapy, with polymorphisms predictive of outcome in some studies of DLBCL and FL [55–58] but not in others [59–63]. FcγR polymorphisms may only be clinically relevant for rituximab monotherapy, or in patients with limited rituximab exposure [64]. The impact of FcγR polymorphisms has not been observed in CLL, for rituximab monotherapy or immunochemotherapy, possibly due to overall impaired effector cell function in CLL [65,66]. These results suggest that disease-specific primary mechanisms of action may underlie mAb-mediated cell death.

Emerging data suggest a "vaccination" effect with anti-CD20 antibodies, whereby cell death enhances dendritic cell maturation and T-cell activation to produce an antilymphoma immune response [67]. "Proof-of-principle" data have demonstrated an increased level of FL idiotype-specific T cells relative to baseline after rituximab treatment in five FL patients [68]. This long-term "vaccination" effect, which may prolong survival, has been demonstrated in mice expressing human CD20 [69]. It appears to be predominantly mediated by Fc–FcγR interactions including FcγRIIIa, with the Fc component required for long-lasting tumor protection in immunocompetent mice [69].

The apparent importance of Fc–FcγR interactions for effector cell-mediated killing of lymphoma cells, as well as other potential effects following mAb treatment, has been a key consideration in the development of next-generation mAbs. Greater understanding of these mechanisms and Fc–FcγR interactions in different clinical contexts may facilitate the optimal use of anti-CD20 mAbs. For

instance, combining rituximab with lenalidomide, an immunomodulatory agent that may stimulate T- and NK-cell cytotoxicity [70], may provide substantially high response rates in previously untreated indolent NHL patients [71]. Given the improved in vitro ADCC and ADCP of obinutuzumab compared with rituximab, further investigations into such combinations are worthwhile. A phase I/II clinical trial (NCT01582776) evaluating combination treatment with obinutuzumab plus lenalidomide is underway.

Obinutuzumab dosing

Preclinical data show that obinutuzumab has superior efficacy over rituximab at the same dose of mAb, indicating that enhanced clinical efficacy may not be simply related to the higher mAb dosing of obinutuzumab (1000 mg) compared with standard rituximab dosing (375 mg/m^2 in NHL and 375 then 500 mg/m^2 in CLL). No increase in response was observed with an increased dose of rituximab monotherapy (from 375 to 500 mg/m^2) in a phase II study in aggressive NHL [72], and although the rituximab 500 mg/m^2 dose in CLL was based on a dose–response relationship [73], the addition of two extra doses of rituximab to each cycle of standard rituximab with fludarabine and cyclophosphamide (FC) in previously untreated CLL did not increase efficacy [74]. The dose for obinutuzumab was based on dose-escalation studies and optimized via pharmacokinetic modeling [75,76].

Clinical experience with obinutuzumab

The improved in vitro and preclinical activity *vs.* type I mAbs, led to obinutuzumab becoming the first glycoengineered type II anti-CD20 mAb in clinical development.

Early-phase clinical trials in NHL

Obinutuzumab elicited responses in rituximab-refractory disease in three phase I trials [77–79]. In one study, 21 patients with relapsed/refractory NHL were administered eight 21-day cycles of obinutuzumab monotherapy at doses ranging from 50/100 to 1200/2000 mg. There were nine responders (five complete responses [CR], four partial responses [PR]s) [77]. In another study, 22 patients, including five with CLL, received four infusions of obinutuzumab 200 to 2000 mg weekly for 4 weeks, with maintenance in responding patients. Five patients achieved PR and 12 stable disease at the end of induction; eight patients received maintenance therapy, during which three patients experienced an improved response [78]. In both trials, investigators reported that obinutuzumab did not induce significant activation of the complement cascade [77,78]. In a Japanese dose-finding study, seven of 12 patients with relapsed/refractory NHL experienced responses to obinutuzumab monotherapy (2 CR and 5 PR) [79].

Adverse events (AEs) were similar in nature to those observed for other anti-CD20 antibodies. Infusion-related reactions (IRRs) were common at first infusion, with few grade 3 or 4 events, and no other specific patterns that could be attributed to obinutuzumab [77,78]. A few patients with MCL experienced rapid depletion of circulating B cells, resulting in clinically significant tumor lysis syndrome. Overall, five patients experienced grade 3 or 4 neutropenia, which resolved with or without growth factor administration [78].

Phase II trials of obinutuzumab in NHL

Phase II studies of obinutuzumab monotherapy in patients with relapsed/refractory indolent NHL ($n = 40$) or aggressive NHL ($n = 40$) have been conducted [80,81]. Based on pharmacokinetic observations in phase I [80], two dose regimens of obinutuzumab were evaluated: 1600/800 mg (1600 mg of obinutuzumab infused on days 1 and 8 of cycle 1 and 800 mg infused on day 1 of cycles 2–8) and 400/400 mg (400 mg given on days 1 and 8 of cycle 1, and then every 3 weeks for seven further cycles) [81]. The 1600/800-mg regimen achieved a response rate of 55% in indolent NHL and 32% in aggressive NHL, whereas the response rates for the 400/400-mg regimen were 17% in indolent NHL and 24% in aggressive NHL. The response rate in rituximab-refractory patients receiving the 1600/800-mg dose was 50% (5/10) for indolent NHL [81] and 33% (4/12) for aggressive NHL [80]. Of the 40 patients with heavily pretreated aggressive NHL (median of three prior treatments), 63% of whom were rituximab-refractory, obinutuzumab yielded a best overall response rate (ORR) of 32% in patients with DLBCL (8/25) and 27% in those with MCL(4/15) [80,81]. ORRs for obinutuzumab were comparable with those for rituximab (30%) in a less heavily pretreated, rituximab-naïve population [82]. These results also compare favorably with those reported for other type I antibodies; for example, ofatumumab achieved an ORR of 11% in a similar patient population with relapsed/refractory DLBCL previously exposed to rituximab [83].

In the randomized phase II GAUSS trial, 175 patients with heavily pretreated, relapsed indolent NHL received four weekly infusions of obinutuzumab 1000 mg or rituximab 375 mg/m^2. All patients had previously responded to rituximab. The end-of-induction investigator-assessed response rates for obinutuzumab and rituximab in patients with FL were 43.2% and 38.7%, respectively, and the CR rates were 10.8% and 6.7%, respectively. A central independent review of responses reported ORRs of 43.2% and 28.0% for obinutuzumab and rituximab, respectively; however, no differences in PFS were observed, albeit that the study was not powered to determine such differences. The toxicities of both treatments were similar, but more patients presented with cough in the obinutuzumab arm (10% *vs.* 1%) and a greater proportion experienced IRRs (any grade, 72% *vs.* 49%; grade 3 or 4, 11% *vs.* 5%) [84].

Use of obinutuzumab in combination with chemotherapy has also been explored. In the phase Ib GAUDI study, patients with relapsed/refractory FL were assigned to either CHOP (six to eight cycles every 3 weeks) or FC (four to six cycles, every 4 weeks) per standard institutional practice and then randomly assigned to either obinutuzumab 1600/800 mg or 400/400 mg. At the end of induction, 96% of patients receiving obinutuzumab plus CHOP and 93% of those receiving obinutuzumab plus FC responded. Of the 14 rituximab-refractory patients, all experienced at least a PR. The most common treatment-related AE was IRR; most were grade 1 or 2 [85].

Early-phase clinical trials in CLL

In a phase I trial, 13 patients with heavily pretreated relapsed/refractory CLL received eight 21-day cycles of obinutuzumab (400–200 mg). Reduced B-cell counts were apparent from first dose and maintained throughout treatment. Eight patients (62%) achieved PR. However, the end-of-treatment response in phase II was lower; 25% (4/16) achieved PR [86], a difference attributed to differences in tumor burden between phases; serum concentrations of type I mAbs are lower in patients with higher tumor burden, which is associated with poorer prognosis [87–90]. Overall, of the 12 patients who responded to obinutuzumab, nine had a limited baseline tumor burden (sum of product of diameter below 2000 mm^2) [86].

In the phase II GAGE study evaluating 1000 *vs.* 2000 mg obinutuzumab, activity was seen at both doses (ORR 49% and

67%, respectively; $p = .08$). The most common AE was IRRs, and all Grade 3–4 IRRs were confined to cycle 1 [91].

In the phase Ib GALTON trial, measuring preliminary efficacy and safety of obinutuzumab in combination with bendamustine or FC, ORR was 90% and 62% respectively. IRRs were again the most common AE (88% patients; grade 3–4 20%) [92].

Phase III trials in CLL

Elderly patients with CLL and those with comorbidities are routinely treated with chlorambucil monotherapy, as no conclusive evidence exists for the superiority of other currently available options [93]. The phase III CLL11 trial investigated patients with previously untreated comorbid (Cumulative Illness Rating Scale score >6) CLL and compared the safety and efficacy of six 28-day cycles of combination treatment with either obinutuzumab (1000 mg on days 1, 8, and 15 of cycle 1 then day 1 of cycles 2 to 6 [a protocol amendment allowed the administration of the first dose of obinutuzumab over 2 days]) or rituximab (375 mg/m^2 on day 1 of cycle 1 then 500 mg/m^2 on day 1 of cycles 2–6) plus chlorambucil (0.5 mg/kg on days 1 and 15 of each cycle), with chlorambucil monotherapy [94].

Stage 1 investigated the benefit of adding obinutuzumab or rituximab to chlorambucil; 118 patients were randomly assigned to chlorambucil alone, 238 to obinutuzumab plus chlorambucil, and 233 to rituximab plus chlorambucil. In stage 1, 77.3% of patients receiving obinutuzumab plus chlorambucil responded to treatment (22.3% CR) *vs.* 31.4% of patients receiving chlorambucil only (0% CR). A higher end-of-treatment ORR was also observed for rituximab plus chlorambucil (65.7%; 7.3% CR) *vs.* chlorambucil only (31.4%; 0% CR). Interestingly, minimal residual disease (MRD)-negativity (by polymerase chain reaction) was not observed in the chlorambucil-only arm, but was achieved with obinutuzumab plus chlorambucil (31.1% peripheral blood and 17.0% bone marrow) and in a small percentage of those treated with rituximab plus chlorambucil (2.0%, peripheral blood; 2.8%, bone marrow) [94]. At a median observation time of 23 months, investigator-assessed median PFS was significantly greater with obinutuzumab plus chlorambucil *vs.* chlorambucil alone (26.7 *vs.* 11.1 months; hazard ratio [HR], 0.18; $P < 0.0001$) [94]. Similarly, compared with chlorambucil alone, rituximab plus chlorambucil was associated with significantly prolonged investigator-assessed median PFS (16.3 *vs.* 11.1 months; HR, 0.44; $P < 0.0001$) [94].

Stage 2 randomized additional patients to compare obinutuzumab with rituximab when combined with chlorambucil; 333 patients received obinutuzumab plus chlorambucil, and 330 received rituximab plus chlorambucil. The end-of-treatment ORR was significantly greater with obinutuzumab plus chlorambucil *vs.* rituximab plus chlorambucil (78.4% *vs.* 65.1%, $P < 0.0001$), with a threefold increased proportion of patients with CR (20.7% *vs.* 7.0%). The percentages of patients negative for MRD in the bone marrow (19.5% *vs.* 2.6%, $P < 0.0001$) and blood (37.7% *vs.* 3.3%, $P < 0.0001$) were also significantly greater for obinutuzumab plus chlorambucil. After a median observation time of 18.7 months, median PFS was significantly prolonged with obinutuzumab plus chlorambucil relative to rituximab plus chlorambucil (26.7 *vs.* 15.2 months; HR, 0.39; $P < 0.0001$). OS was significantly improved in the obinutuzumab plus chlorambucil arm compared with chlorambucil monotherapy (HR for death, 0.41; $P = 0.002$), with no significant difference shown for the rituximab plus chlorambucil arm *vs.* chlorambucil monotherapy (HR, 0.66; $P = 0.11$). However, there was no significant difference in OS between the combination therapy arms (HR, 0.66; $P = 0.08$) [94]. In a later update to this study, the PFS advantage for obinutuzumab plus chlorambucil compared with rituximab plus chlorambucil (median PFS 29.2 *vs.* 15.4 months; HR, 0.40; $P < 0.001$) was confirmed, although OS data were immature at the time of reporting, there were only 45/333 deaths in the obinutuzumab plus chlorambucil arm and 63/330 in the rituximab plus chlorambucil arm [95].

The COMPLEMENT 1 study, which compared ofatumumab plus chlorambucil with chlorambucil alone in untreated CLL reported a statistically significant improvement in median PFS (22.4 *vs.* 13.1 months; $P < 0.001$) in 221 patients randomized to ofatumumab (300 mg day 1 and 1000 mg day 8, then 1000 mg day 1 of each 28-day cycle) plus chlorambucil (10 mg/m^2 on days 1–7 of each cycle) compared with 226 patients randomized to chlorambucil alone [96].

Safety profile of obinutuzumab

In stage 1 of the phase III CLL11 study, grade $\geqslant 3$ IRRs occurred in 21% of patients who received obinutuzumab, all at first infusion [97]. In stage 2, the incidence of grade $\geqslant 3$ IRRs was higher with obinutuzumab plus chlorambucil *vs.* rituximab plus chlorambucil (20% *vs.* 4%) [94]. Interestingly, this difference was greater than that observed for grade $\geqslant 3$ IRRs between obinutuzumab and rituximab when given as monotherapy for relapsed indolent NHL (11% *vs.* 5%, respectively) [78]. The higher affinity of obinutuzumab for FcγRIII binding to CD20 on peripheral cells may lead to stronger FcγR activation and subsequent target mediated cytokine release, particularly in first-line treatment of patients with high peripheral CLL counts. Indeed, CLL patients with higher CD20 expression, FcγRIII expression, or expressing the higher affinity FcγRIII genotype are at increased risk of developing IRRs [98]. A significant decrease in circulating B cells and increase in the pro-inflammatory cytokines IL-6, IL-8, TNF-α, and IFN-γ has also been shown following the first infusion of obinutuzumab [99], which may account for the increased incidence of IRRs. Preliminary safety data from GREEN showed fewer grade $\geqslant 3$ IRRs with a split initial dose of obinutuzumab over 2 days in Cycle 1 (25 mg on Day 1 and 975 mg on Day 2), and lower infusion rate (the Day 1 dose was given at 12.5 mg/h); however, discontinuation levels were similar to previously reported studies [100]. IRRs were well managed with acetaminophen/paracetamol, antihistamine (30 min prior to first dose and for subsequent doses if required) and steroid (prednisone 100 mg iv at least one hour before the Cycle 1 obinutuzumab dose on Day 1 and Day 2) premedication. Although stage 1 data from the phase III CLL11 study showed an increased incidence of grade $\geqslant 3$ neutropenia among patients receiving obinutuzumab plus chlorambucil *vs.* chlorambucil alone (35% *vs.* 16%), the rate of grade $\geqslant 3$ infection was slightly higher with chlorambucil monotherapy (14% *vs.* 11%) [94]. Similar trends were observed in stage 2, with slightly higher rates of grade $\geqslant 3$ neutropenia and thrombocytopenia for obinutuzumab plus chlorambucil *vs.* rituximab plus chlorambucil (33% *vs.* 28% and 11% *vs.* 28%, respectively) and similar rates of grade $\geqslant 3$ infection (12% *vs.* 14%) [94]. The observed incidence of neutropenia may be related to enhanced neutrophil consumption due to ADCP [41]. The potential longer-term significance of this increased neutropenia remains to be determined and further careful observation is required.

Will obinutuzumab be effective in rituximab-refractory disease?

Rituximab resistance is now an important challenge in the treatment of B-cell malignancies. There are numerous, physiologically diverse mechanisms by which rituximab resistance can develop, including tumor-specific mechanisms (reduced tumor penetration, impaired mAb binding, loss/downregulation of CD20, resistance of tumor cells to mAb-effector mechanisms) and factors

related to patient physiology (increased mAb metabolism, impaired immune effector cell recruitment or function) [101].

Loss of the CD20 antigen from lymphoma cells was first reported by Davis et al. [102], and it was subsequently shown that B cells with significantly reduced CD20 levels could emerge following rituximab infusion in CLL patients [103]. CD20 loss contributes significantly to the lack of response to rituximab in some patients [104], and occurs by at least two mechanisms. The first involves the "shaving" of rituximab/CD20 complexes from the cell surface, mediated by phagocytic cells when immune effector mechanisms become saturated by high levels of circulating target antigen [105,106]. A second mechanism involves internalization of CD20 into lysosomes via endocytosis; and occurs with type I, but not type II, anti-CD20 mAbs [107]. Modulation of CD20 location may underlie the reduced clinical efficacy of type I mAbs in CLL and MCL [26,107]. Obinutuzumab monotherapy has displayed significant activity in rituximab-refractory disease in phase II studies [80,81,108], which justified a randomized phase III study (GADOLIN, NCT01059630) investigating whether obinutuzumab plus bendamustine confers clinically meaningful benefit *vs.* bendamustine monotherapy in rituximab-refractory indolent NHL; this study was reported early as the primary endpoint had been met at a pre-planned interim analysis.

Conclusions

Obinutuzumab is a glycoengineered, type II anti-CD20 mAb with different mechanisms of action to rituximab, including increased induction of direct cell death and enhanced ADCC/ADCP. Preclinical data show that obinutuzumab has superior efficacy over rituximab at the same dose of mAb.

In CLL11, no relevant induction of MRD negativity was achieved with the addition of rituximab to chlorambucil for CLL, as was apparent with the addition of rituximab to FC in the CLL8 study [109]. Thus, the markedly increased MRD negativity with G-Clb *vs.* R-Clb in CLL11 argues against a sole dose effect, but infers a different biological mechanism of disease eradication in CLL. Owing to differences in the chlorambucil schedules used, in the absence of a direct comparison of the type I anti-CD20 mAb ofatumumab with the type II mAb obinutuzumab, it is not possible to determine which is the superior mAb. Preclinical insights regarding CD20 expression and modulation imply that the mechanism of action of type II anti-CD20 mAbs is more advantageous than that of type I mAbs in B-cell malignancies. To date, clinical data suggest that differences in activity may be more pronounced in B-cell malignancies such as CLL and MCL, albeit numbers of MCL are low. It is possible that differences may be seen across different B-cell malignancies and no signal of superiority has yet been seen in FL; where there was no significant PFS increase for obinutuzumab *vs.* rituximab in the GAUSS study [84].

Obinutuzumab underpins a number of ongoing phase III clinical trials in patients with untreated, and rituximab-refractory, indolent NHL. In particular, direct comparisons of obinutuzumab-CHOP *vs.* R-CHOP in untreated patients with CD20-positive DLBCL, and obinutuzumab or rituximab plus CHOP, CVP (cyclophosphamide, vincristine, and prednisone), or bendamustine in untreated advanced indolent NHL will provide further evidence of the potential for improved outcomes with obinutuzumab in other B-cell malignancies.

In CLL, therapeutic alternatives and scheduling options are complex, with high clinical activity noted for many novel agents. Many trial groups are considering "mild" short-acting chemotherapy to debulk the tumor, before administering at least two or three of the best novel agents: for example, ABT-199 plus obinutuzumab, ibrutinib plus obinutuzumab, or idelalisib/ibrutinib/ABT-199 [110]. These combinations will need rigorous testing in well-designed clinical trials assessing MRD status.

Further phase III clinical trial data are eagerly awaited including obinutuzumab or rituximab plus chemotherapy in patients with previously untreated indolent NHL (NCT01332968); bendamustine with or without obinutuzumab in rituximab-refractory indolent NHL (NCT01059630); obinutuzumab or rituximab plus chemotherapy in first-line DLBCL (NCT01659099, NCT01287741).

Ongoing trials will provide evidence of whether patient outcomes in other B-cell malignancies can be further improved by the introduction of this novel mAb.

Conflicts of interest

TI has served as a consultant and has participated in a Speaker's Bureau for F. Hoffmann-La Roche, and has received research finding from Glycart.

CK is an employee of F. Hoffmann La Roche and holds stock and patents.

LHS has received honoraria from F. Hoffmann-La Roche/Genentech, Celgene, Gilead, Amgen, Janssen and Lundbeck, and has served as a consultant for F. Hoffmann-La Roche/Genentech.

AD has received honoraria from F. Hoffmann-La Roche, Takeda and Gilead; has served as a consultant for F. Hoffmann-La Roche and Gilead; has received research funding from F. Hoffmann-La Roche, Gilead, Pfizer, Bayer and Celgene; and has received travel, accommodations or expenses from F. Hoffmann-La Roche.

GS has served as a consultant and received honoraria from Gilead, Janssen, F. Hoffmann-La Roche, Mundipharma and Celgene, and has received research funding from F. Hoffmann-La Roche.

GC has received honoraria from F. Hoffmann-La Roche, Celgene, Mundipharma and Janssen, has served as a consultant for F. Hoffmann-La Roche, and has received travel, accommodations or expenses from BMS, Novartis and Celgene.

Contributors

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